

REGARDING THE LENGTH AND EXTENT OF THE HUMAN MEDULLA SPINALIS

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Most of our modern text-books on human anatomy state that the spinal medulla is variable in length and in extent within the vertebral canal and that there is no definite relation between the length of the spinal medulla on the one hand and the length of the vertebral column and the height of the individual on the other. It was with these well known facts in mind that the study of this series of 234 cases was undertaken.

The observations about to be reported were made on the cadaver in the dissecting room. The material had been prepared for dissecting purposes by the method described by Professor Streeter in volume 5 of *The Anatomical Record*. During the regular course in Gross Anatomy as given in this department the superficial and deep muscles of the back are identified and removed thereby exposing the entire length of the vertebral canal. The neural arches are then removed. The cut pedicles of the vertebrae and the posterior root ganglia of the spinal nerves are exposed. The dural and arachnoid membranes are opened by an incision along the mid dorsal line bringing into view the spinal medulla and the root filaments of the spinal nerves. The relation of the neural segments of the spinal medulla with the vertebrae is readily determined.

Because of the impossibility to determine with accuracy, macroscopically, the lowest point to which the nervous elements of the spinal medulla extend and the beginning of the filum terminale, the attachment of the lowest root filaments of the fourth sacral nerve was taken as the lowest point in all measurements. To avoid error the number of ribs and vertebrae were taken in all cases. The cadaver was placed in the prone position on a

TABLE I

Shows the length of the medulla spinalis and its level of termination in the vertebral canal; measurements given in centimeters

EXTENT OF SPINAL MEDULLA	MALE, WHITE						FEMALE, WHITE						NEGRO					
	Cord lengths			Height of subject			Cord lengths			Height of subject			Cord lengths			Height of subject		
	Per cent	No.	Ave.	Extremes	Extremes	Ave.	Per cent	No.	Ave.	Extremes	Extremes	Ave.	Per cent	No.	Ave.	Extremes	Extremes	Ave.
Middle of 12 T. vert.....	1.0	2	41.7	39 to 44	160 to 180	170.0							1	10	42.0			160.0
Lower border of 12 T. vert.	6.5	13	43.5	40 to 46	164 to 178	168.9	2	8	39.25	38 to 40	150 to 162	156.0	4	40	41.1	38 to 45	156 to 181	166.4
Upper border of 1 L. vert...	15.0	30	44.5	41.5 to 47	159 to 182	171.5	4	16	40.6	38 to 44	152 to 164	160.5	1	10	41.0			172.0
Middle of 1 L. vert.....	29.1	58	44.0	37 to 49	151 to 186	169.3	5	20	40.5	39 to 41	156 to 170	162.2	1	10	49.25			177.5
Lower border of 1 L. vert...	20.1	40	45.3	39 to 52	149 to 196	170.6	6	24	40.75	40 to 45	151 to 166	155.5	2	20	45.5			171.5
Upper border of 2 L. vert.	13.5	27	45.1	41 to 49	156 to 187	169.2	3	12	44.1	43 to 45	158 to 162	160.5						
Middle of 2 L. vert.....	13.5	27	46.2	40 to 51	135 to 187	167.1	4	16	45.0	44 to 47	160 to 172	169.0						
Lower border of 2 L. vert...	1.0	2	48.5	47 to 51	168 to 172	170.0	1	4	45.3			150.0	1	10	47.5			167.0
Total.....	99.7	199	44.79			169.6	25	100	41.8			159.4	10	43.4				168.4

flat table top. The length of the spinal medulla here given represents the distance between the lower border of the foramen magnum and the lowest filaments of the fourth sacral nerve roots.

By consulting table 1 it will be seen that these observations have been classified according to sex and race. In about 77 per cent of the white male subjects the lowest point of the spinal medulla was found to extend between the level of the upper border of the first and the upper border of the second lumbar vertebrae. In two cases the spinal medulla extended only to the middle of the twelfth thoracic vertebra and in two others it terminated on a level with the lower border of the second lumbar vertebra.

There is a gradual average increase in the length of the spinal medulla with the increase in extent down the vertebral canal. However, spinal medullae of average length may terminate high in the canal, as in one of our series where a spinal medulla 44 cm. in length extended to the level of the middle of the body of the twelfth thoracic vertebra. Short spinal medullae may extend relatively low in the vertebral canal, as in a case where a spinal medulla 37 cm. in length extended to the level of the middle of the body of the first lumbar vertebra. As a rule the long spinal medullae extend relatively low in the vertebral canal. A spinal medulla 50 cm. in length terminated at the level of the lower border of the second lumbar, and one 52 cm. in length extended to the lower border of the first lumbar vertebra. It will be seen also that the length of the spinal medulla and the height of the individual bear no definite relation to one another.

Observations were made on a series of 25 females, in about 72 per cent of which the spinal medulla extended between the level of the upper border of the first and the upper border of the second lumbar vertebrae. As in the male series the short spinal medullae terminated as a rule at a higher level in the vertebral canal than the longer ones. In this group spinal medullae 38 cm. and 40 cm. extended to the level of the lower border of the first lumbar vertebra. As in the male series there was a gradual average increase in the length of the spinal medulla with its

extent down the vertebral canal and there also appeared to be no relation between the length of the spinal medulla and the height of the individual.

In the third group of 10 negro subjects the spinal medulla in 90 per cent did not extend below the level of the lower border of the first lumbar vertebra. As in the series of white subjects there is a gradual average increase in the extent of the spinal medulla down the vertebral canal with the increase in length of the former. A spinal medulla 42 cm. in length terminated at the level of the middle of the body of the twelfth thoracic vertebra, one 49 cm. in length reached to the level of the middle of the body of the twelfth thoracic vertebra, one 49 cm. long reached to the level of the middle of the first lumbar vertebra and a spinal medulla 47.5 cm. in length extended to the lower border of the second lumbar vertebra.

TABLE II.

Shows the relative lengths of medulla spinalis and vertebral column

NO. OF CASES	AVERAGE LENGTH OF VERTICAL COLUMN	AVERAGE LENGTH OF SPINAL CORD
	<i>cm.</i>	<i>cm.</i>
2	68	40.0
4	72	44.5
8	76	45.1
4	81	49.0

In 46 cases the length of the spinal column was also taken to determine if there was any definite ratio between the length of the spinal medulla and the vertebral axis. The length of the vertebral column was determined by taking the distance between the lower border of the foramen magnum, and the tip of the coccyx. By referring to table 2 it will be seen that the length of the spinal medulla is not in definite proportion to the length of the vertebral column in these cases, but that short vertebral columns were always associated with short spinal medullae and that long vertebral columns with long spinal medullae.

By comparing the results of this investigation with those of earlier reports it will be seen by consulting table 3 that the average length of the spinal medulla in the male (44.79 cm.) in our series

TABLE III
Gives comparisons between earlier observations and the authors cases

OBSERVER	DATE	MALES					FEMALES						
		No. of cases	Av. height in cm.	Av. length of v. col. in cm.	Av. length of cord in cm.	Limits of variability of cord levels	Limits of variability of cord lengths in cm.	No. of cases	Av. height in cm.	Av. length of v. col. in cm.	Av. length of cord in cm.	Limits of variability of cord levels	Limits of variability of cord lengths in cm.
Pfitzner.....	1884	6			46.8			1			43		
Moorhead ¹	1895	17	167.5	58.3	45	5 mm. above lower border 12 T. vert. to mid. 2 L. vert.	43.5 to 46.5	23	157.5	55.3	43.7	Mid 1 L. vert. to lower border 2 L.	39.5 to 47
Thompson.....	1895	115				Mid. 12 T. vert. to upper border 3 lumbar.		83				5 mm. below 12 T. vert. to 5 mm. below 2 L.	
Author.....		199	169.6		44.79	Mid. 12 T. vert. to lower border 2 L. vert.	37 to 52	25	159.4		41.8	Lower border 12 T. vert. to lower border 2 L.	38 to 47

¹ Moorhead's cases are included in Thompson's collective report.

is somewhat shorter than that given by Pfitzner and Moorhead; that the limits of variability of cord lengths is greater and that the limits of variability of cord levels in the vertebral canal are about the same as that of the earlier writers.

It will be seen, also that the average length of the spinal medulla in the female (41.8 cm.) is less than previously given and that the limits of variability of cord lengths and levels are nearly the same.

Finally, we conclude from the foregoing:

1. That there is no relation between the length of the spinal medulla and the height of the subject although tall individuals usually have a long spinal medulla and short individuals a short spinal medulla.

2. That there is no definite ratio between the lengths of the vertebral axis and the spinal medulla. However, short vertebral axes always enclosed short spinal medullae and long columns long spinal medullae.

3. The lowest point to which the spinal medulla was found to extend was to the level of the inferior border of the second lumbar. The highest level at which it was found to end was at the level of the middle of the body of the twelfth thoracic vertebra.

4. The average length of the spinal medulla for the male was 44.79 cm., for the female 41.8 cm. and for the negro 43.4 cm.

LITERATURE CITED

- MOORHEAD, J. H. 1895 Jour. Anat. and Phys., vol. 29, pp. 48-52.
PFITZNER, W. 1884 Über Wachstumsbeziehungen zwischen Rückenmark und Wirbelkanal. Morph. Jahrb., Bd. 9, S. 99-116.
THOMPSON, A. 1895 Fifth annual report of the committee of collective investigation, etc. Jour. Anat. and Phys., vol. 9, pp. 46-48.

STUDIES ON CELL DIVISION IN THE ALBINO RAT (MUS NORVEGICUS, VAR. ALBA)

II. EXPERIMENTS ON TECHNIQUE, WITH DESCRIPTION OF A METHOD FOR DEMONSTRATING THE CYTOLOGICAL DETAILS OF DIVIDING CELLS IN BRAIN AND TESTIS

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ONE TEXT FIGURE AND TWO PLATES

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INTRODUCTION

This paper deals with a series of experiments on technique by which a method has been developed for demonstrating the details of mitosis in the central nervous system and testis of the albino rat without producing distortion of the other parts of the tissues. The work was begun because of difficulty experienced

in positively identifying dividing cells while conducting experiments on influencing the rate of cell division in the cerebellum of the albino rat. In the external granule layer of a sick individual seven days old, I found a large number of peculiar cells which oftentimes resembled certain phases of dividing cells. The tissue had been fixed in Carnoy's fluid and treated according to the method previously used in my study determining the duration of cell division in the central nervous system of the albino rat (Allen '12). At Professor McClung's suggestion I attempted to secure a technique which would insure the cytological details desired.

King ('10), after a thorough and exhaustive set of experiments on various standard fixatives for brain tissue, showed that for general histological purposes Ohlmacher's fixing fluid followed by double imbedding with celloidin and paraffin gave the best results. This method, however, fails to prevent the chromosomes from fusing, and consequently was inadequate for the purpose in hand.

My experiments grew to a series of tests dealing with each step in the preparation of sections, finally resulting in a method which not only clearly differentiates dividing cells in all stages, but also prevents fusing, or 'clumping,' of the chromosomes, a distinct gain in the technique for mammalian tissue. Other workers in the Zoological Department of the University of Pennsylvania are experimenting on other tissues. The results of their work will also be published, so that this paper forms one of a series on the problems of technique.

THE SCOPE OF THE EXPERIMENTS

It is needless to record all the details of the work done or of the results obtained. The general plan and scope of the work are as follows: While a few experiments were made on a pure trial and error basis, with the hope that something desirable would result, most of them were carefully planned along the line of modifying approved methods. The success of each experiment was measured by two criteria: (1) the isolation and definition of the

chromosomes and (2) the 'general fixation.' The term 'general fixation' covers the points of shrinkage or swelling of the whole mass, and the degree of distortion of one part or of one tissue. A good general fixation describes the condition of a preparation which has suffered only slight changes in total volume, either by shrinkage or swelling, and in which the individual parts maintain their normal relationship of volume and structure; that is, the connective tissues are not torn, the limiting membranes retain their size and smooth outlines, while the cells show no vacuoles about them nor cytolysis of any kind.

For a long time it looked as if excellence of general fixation would have to be sacrificed to distinctness of the chromosomes, so that I was particularly pleased when the best chromosome differentiation obtained was accompanied by the best general fixation. A comparison of figures 2, 9, 10, 13 and 15 with figures 4, 7, 12, 14 and 17 respectively will show the contrast between poor and good preparations according to the standards just described.

EXPERIMENTS ON FIXATIVES

The first line of investigation followed was that of modifying the proportions of constituent parts of Bouin's fluid, since this fixative had proved so excellent for general purposes. The proportions are given in table 1. The first set of experiments was with the first six modifications shown in table 1. One promising fluid appeared in modification no. 2. It failed, however, upon further trial to yield constant or fully satisfactory results, though it seems to be a better fixative for albino rat tissue than Bouin's combination.

After thorough trial of the picro-formol-acetic combinations as far as no. 14 in the table, the following fluids were used: Flemming's, Worcester's, Lavdowsky's, Carnoy's (often called van Gehuchten's), Ohlmacher's, King's potassium-bichromate-acetic-sublimite, and Tellyesniczky's. Urea was added to each in varying proportions for studying its effects; fixatives were made up in normal salt solution; different temperatures—0°C., room and 30 to 40°C.—and different fixation periods were tried with any

TABLE 1

Showing the various combinations of picric acid, formol, acetic acid, corrosive sublimate, chromic acid and urea employed. Fluids are measured in cubic centimeters; solids, in grams; the percentages are thus indicated

SOLUTION	PICRIC ACID; SATU- RATED AQUEOUS SOLUTION	FORMOL	GLACIAL ACETIC ACID	CORRO- SIVE SUB- LIMATE SATU- RATED; AQUEOUS SOLUTION	SALT; CRYSTALS	UREA; CRYSTALS	CHROMIC ACID; CRYSTALS	WATER
Bouin ¹	75	25	5					
No. 1	75	15	5					
2	75	10	10					
3	75	15	10					
4	75	10	5					
5	75	5	5					
6	75	10	10	5				
7	50	10	10					25
8	90	5	5		0.9	0.5		
9	75	10	10		0.9	1.5		
10	75	15	10		0.9	1.5		
11	75	10	10			0.1		100
12	75	10	10			1.5		300
13	75	25	5			4.0		300
14	75	25	5		0.9	0.5		
15	75	25	5			2.0	1.5	
16	75	20	3			1.0	1.0	
17	75	5	1			1.0	0.5	
18	75	20	7			0.3	0.5	

¹ Lee ('13).

fluid which seemed hopeful on first use. Various strengths of formalin, with and without sugar, and different combinations of acetone and formalin were also tried. See table 2.

A second promising fluid resulted from the addition of a little dilute chrome-acetic mixture (one of the parts of Flemming's fluid) to the Carnoy fluid. The general fixation was excellent and the dividing cells in the brain showed better cytological detail than with anything else tried up to that time. This combination was therefore tested. The constituents were mixed in different proportions; and different temperatures, periods of fixation and quantities of urea tried out. Chromic acid yielded better and better results upon the chromosomes as its percentage was increased, but the mitotic figures were still not satisfac-

TABLE 2

Showing some of the various combinations of fluids used based upon either formol, acetone or Carnoy. The measurements are the same as in table 1

SOLUTION	FORMOL (NEU- TRAL)	ACE- TONE	ALCO- HOL (100 PER CENT)	CHLO- ROFORM	GLACIAL ACETIC ACID	CHRO- MIC ACID; CRYS- TALS	CORRO- SIVE SUBLI- MATE; CRYS- TALS	UREA; CRYS- TALS	SUGAR; CRYS- TALS	WATER
No. 1 } McClendon }	10								2.0	88
2	10							1	1.0	90
3	10								0.5	90
4	1							1	1.0	98
5	2							1	1.0	98
6	5							1	1.0	94
7	2		48					1		50
8	1	90						1		10
9	10	50						1	1.0	40
10	5	50						1	1.0	40
11	10		50					1		45
12	5		50					1	1.0	45
Carnoy			60	30	10					
13			60	30	5	1.0				
14			98			5.0				2
15			60	30	10	1.5		2		
16	5		60	30		1.5		2		2
17			60	30		1.5		2		2
Ohlmacher			80	15	5		Sat- ura- tion			

tory for accurate work when the concentration had reached a point in which the rest of the tissues were torn and distorted worse than the results of Flemming's fluid shown in figure 13. Some of the combinations used are shown in table 2, nos. 13 to 17. Formol and acetic acid both swell tissues. For this reason formol was substituted for the acetic acid in several trials, only one of which is shown in the table (no. 16, table 2).

Finally one half of a seven day brain was put into the combination of chromic acid and Carnoy's fluid which had given the best results, and the other half in some Bouin's fluid to which I added at the same time some chromic acid and urea. The half placed in the latter fluid showed all the good points of general

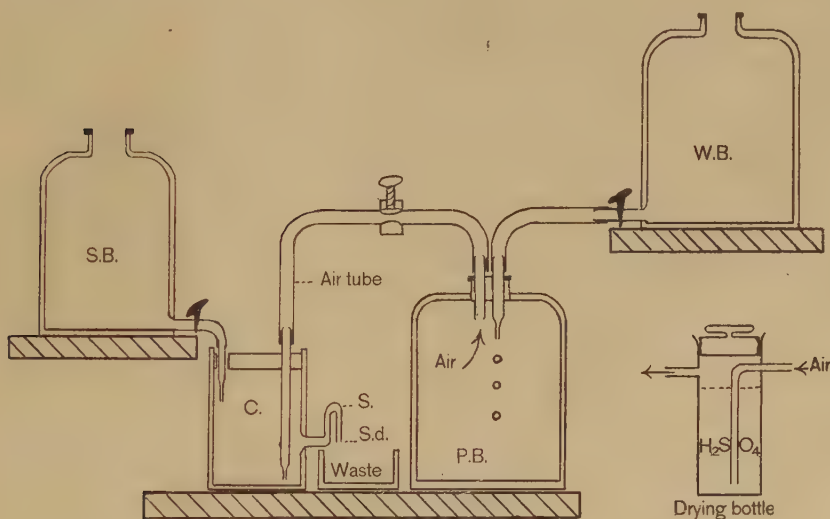
fixation gotten by Bouin's fluid with the addition of well differentiated chromosomes, while the other half was useless in comparison. Further trials of the new fixative (no. 15, in table 1) yielded practically constant results and later when applied to the testis gave the material from which figures 12, 14 and 17 were made. The exact proportions used appear in table 1, no. 15. This fixative will henceforth in this paper be spoken of as the picro-formol chrome-acetic in urea fixative, or for convenience 'B-15.'

DEHYDRATION

The well recognized necessity of a very slow change from a fluid of one density and osmotic pressure to another which differs in these respects, in order that cytolysis may be avoided, led to a consideration of how this change might be easily and effectively accomplished. Professor McClung while working on grasshopper testis found that if the higher grades of alcohol were added drop by drop better results were obtained than by transferring the tissue from water to 35 per cent, 50 per cent, 60 per cent alcohol, etc. To accomplish this purpose he used the principle of capillarity in a cotton cord or a fine glass siphon. This method, however, when applied to more than two or three cubic centimeters of fluid did not insure mixing, the lighter liquid remaining on top of the heavier. Even when the lighter liquid is introduced through a tube to the bottom of the heavier, it rises immediately and accumulates on top of the heavier in an independent layer and remains there for days, as is readily shown by introducing colored alcohol in the manner described. I have found that a flow of air gently and rapidly mixes the fluids of different density. The current may be obtained from a pressure bottle when compressed air is not available in the laboratory. The apparatus devised for this purpose is shown in text-figure 1. It consists of two bottles of equal volume, one for water and the other for air pressures, marked *W.B.* and *P.B.* One of these is of the aspirator type. The remainder of the apparatus is a supply bottle for storing the alcohol or other replacing fluid, *S.B.*, also fitted with bottom stopcock; a container

for the tissue, *C*, fitted with a siphon opening from the side, *S*; a jar for receiving waste; glass and rubber tubing for connections as shown; corks and screw clamps or stopcocks of small bore, as indicated in the figure.

The size of the water and pressure bottles should be two or more liters, in order that little attention need be given the supply of air. A variety of sizes of the container is desirable, since a small amount of tissue needs only a small amount of fluid. For two or three pieces of 0.5 cc. volume a vessel of 30 cc. total



capacity is ample. Shell vials or shallow stender dishes are very suitable. One should avoid narrow, tall containers as handling the tissue in them is difficult. The supply bottle for alcohol may be large or small, depending upon the quantity of fluid needed. Where large quantities of tissue are being dehydrated or changed, several supply bottles will be convenient, one for each kind of fluid that is in use.

If aspirator bottles are not available, ordinary bottles fitted with siphons through their corks may be substituted.

The connections should be made as shown in the diagram. The cork for *P.B.* fits tightly; that for *C* is loose-fitting, or else the

hole for one of the tubes is slightly larger than the tube, to allow for escaping air. The cork for *S.B.* must also fit loosely.

To operate: after seeing that all connections are adjusted, fill the water bottle *W.B.* and open its stopcock slightly until the water begins to drop into the pressure bottle. As soon as the air pressure in this bottle overcomes the pressure of the fluid in *C*, bubbles will emerge from the mouth of the air tube and pass to the top of the fluid. A steady stream of air instead of bubbles is better, and may be produced by drawing out the end of the air tube into almost a capillary and clamping its rubber tube almost shut, thus forcing the air to accumulate under pressure in *P.B.*

Next start the alcohol dropping at the desired rate. The air and alcohol will continue to flow until their supply is exhausted in each case or until stopped by the operator. The siphon will remove the excess of fluid, so that the concentration of the alcohol is steadily rising in *C*. The waste jar must be large enough to accommodate the excess fluid.

A two-liter bottle for *W.B.* if filled will run all night at the rate of a drop a second. When the water has run over into the pressure bottle it is poured back into the water bottle and thus used repeatedly.

When the apparatus is used for higher alcohols or for oils, the air should be dried by passing it through a tube containing calcium chloride or through sulphuric acid. The latter method is easily accomplished by a gas washing bottle, shown at one side in the diagram.

As a concrete illustration, suppose that the tissue is to be raised from water to 75 per cent alcohol. It is placed in the container *C* and covered with the fixing fluid or with whatever fluid it may be in at the time. The air and alcohol are set flowing as previously described. Sometimes a difficulty appears in connection with the stopcocks or pressure clamps. Stoppage will occur in the rubber tubing so clamped after the clamp has been on for some days. Consequently the clamp must be moved occasionally. The stopcocks need turning wide open occasionally and then readjusted to the desired rate of flow.

The rate of dropping is affected by the pressure of the water above the stopcock in the water bottle, this pressure varying directly with the quantity of water. A little experimenting will usually suffice to regulate the flow satisfactorily.

If the tissue is very delicate and is seen to be moving about in the container, the rate of air admission may be slowed or a glass slide interposed between the air and the tissue. This should fit loosely enough to allow of free interchange of liquids about its edges but to prevent rapid currents at the same time. Another excellent scheme is to put the tissue into a smaller bottle, the sides of which have been perforated with numerous holes. This receptacle prevents the air currents from forcibly disturbing the tissue while at the same time it permits very free circulation of the fluids.

The siphon may be adjusted to discharge constantly, once it has started, or intermittently. If its discharge is at the same level as the point where it leaves the container (as shown in the cut), the flow will be constant. If the descending arm is longer than the ascending arm, thus lowering the discharge point, the flow will be intermittent.

By measuring the amount of water in the container at the beginning, one can readily calculate how much alcohol of a given strength will be required to bring the fluid in the container up to 70 per cent, or any other percentage desired. This quantity can be put into the supply bottle *S.B.*

The percentage of alcohol to be used at first varies somewhat with conditions. If the tissue is small compared with the volume of fluid in the container, 95 per cent may be used. On the other hand, if the quantity of fluid is small compared with the tissue it is better to start with 50 per cent, or 70 per cent and continue this until the tissue is in 50 per cent when 95 per cent may be run in. The new fluid may be introduced with constantly increasing speed as its concentration rises in the container.

The same apparatus is very convenient for washing out picric acid or corrosive sublimate, as the current of air hastens the process considerably, since the agitation constantly removes the layer of fluid which exudes from the tissue, and the interchange

is thus not dependent solely upon diffusion of fluids. If desired, alcohol of the same strength may be kept running into the container from the supply bottle; or the fluid in the container may be changed at intervals.

For passing to the higher alcohols and clearing oils, the same apparatus is employed in the same way as for bringing the tissue up to 70 per cent.

THE USE OF ANILIN IN PLACE OF THE HIGHER ALCOHOLS

While carrying on these experiments at Woods Hole, Dr. E. E. Just called my attention to Suchanek's ('90) use of distilled anilin oil as a substitute for the higher alcohols, and spoke of his own success in its use on heteronereis eggs. I tried it after 75 per cent alcohol. It produced no appreciable change in the volume of the tissue even if left in it for twenty-four hours, and I have since employed it entirely except after a Flemming fixation. Tissue so fixed is turned very black by it, even though previously very thoroughly washed.

Anilin does not mix with paraffin but is readily followed by any clearing oil. I have had no difficulty with its use. On the other hand, it seems to improve the tissue for sectioning and staining. By one distillation (the distillation point is 184°C.) anilin becomes a clear, pale-yellowish fluid. This keeps well if stored in the dark. The Kahlbaum anilin comes a pale red and can be used without distillation, but may darken the tissue somewhat.

King ('10) found that double imbedding prevented shrinkage of the nerve cell body. She says:

When a brain that has been fixed in Ohlmacher's solution is imbedded in paraffin after being cleared with chloroform or with any other substance commonly used for this purpose, there is invariably a shrinkage of the cell body, . . . and a condensation or vacuolization of the cytoplasm. If, however, the brain is imbedded in celloidin-paraffin, . . . there is no shrinkage evident anywhere in the cell.

referring to large cells in the cerebral cortex. I am confident that the use of anilin obviates this difficulty, and consequently avoids the shrinkage due to the higher alcohols essential to the

use of celloidin, and furthermore gives perfectly flat sections. The photographs in figures 3 and 4 are typical of the appearances produced by my method of treatment. Neither large nor small cells show vacuolization of cytoplasm nor shrinkage of the nucleus.

CLEARING

Xylol, as is well known (Lee '13) shrinks tissues considerably. I experimented with chloroform, oils of cedar, cassia, wintergreen and bergamot. Each produced less shrinkage than xylol. The effects in each case were measured on the whole mass of tissue, on the smoothness of outline of the individual tubules of the testis, and on nerve cells. The least shrinkage occurred with bergamot; wintergreen is a close second. Cassia (cinnamic aldehyde) seemed excellent for the testis, but since this does not mix with paraffin it must be followed by a paraffin solvent. It has the advantage of mixing with 85 per cent alcohol freely, and it is therefore useful after Flemming to avoid the higher alcohols. The pure synthetic preparations of all the oils are better than the natural products. The following illustrations serve to show the extent of shrinkage during the clearing process.

	cc.
Volume of brain when put into xylol.....	0.65
Volume of brain 2½ hours later.....	0.60
Volume of brain 23 hours later.....	0.50

The loss in this case was 23 per cent. In some cases it was as high as 25 per cent.

The effects of bergamot oil were compared with those of a special cedar oil which has the merit of mixing with alcohol in any proportion. I passed the halves of the same cerebellum through these oils respectively, and then measured ten undeveloped Purkinje cells from each preparation, carefully selecting those cut in the same way. Drawings were made with the aid of the camera lucida. The mean diameter of the ten fixed in bergamot oil was 9 units; that of the ten fixed in the special cedar oil was 8.1 units, a loss of 10 per cent. The diameters of the latter were consistently smaller throughout.

Testis fixed in Flemming seems less susceptible to change in volume at this stage in the technique than when fixed in one of the picro-formol-acetic combinations, an indication that this fixative (Flemming) has brought the tissue more nearly to its limit of shrinkage.

DETAILED STATEMENT OF THE METHOD

Formula for the fixative. (B-15; table 1, no. 15)

	cc.
Picric acid, saturated aqueous solution.....	75
Formol.....	25
Glacial acetic acid.....	5

In this fluid dissolve at time of fixing 1.5 gram of chromic acid in crystals, and then 2 grams of urea in crystals.

Fixation. Heat the fixative to 37° or 38°C., place the tissue in it and hold at that temperature. Pieces of brain 0.5 cc. in volume fix in one hour; pieces of young testis of the same or less volume require a little longer. The adult testis should be given two to three hours, depending on the age of the animal.¹ The external membranes of the tubules and the connective tissues become, with age, progressively less easy to penetrate. Plenty of fixing fluid is used. It is agitated several times during the fixation. To secure quick penetration of the testis it is necessary to cut the outer covering (the tunica albuginea), and in addition snip the tubules apart with scissors.

A low temperature paraffin bath is convenient for keeping the fluid at the desired temperature.

Dehydration. The fluid and contained tissue are cooled to room temperature and slow, gradual dehydration begun with alcohol as described previously (p. 578). The rate of dropping should be about one drop per second unless the quantity of fluid is very small, when it should be less rapid.

After this fixative, it is desirable to bring the tissue up to 75 per cent within an hour (for pieces 0.5 cc. in volume) if the tissue is soft; harder tissues need a little more time.

¹ The young mature testis is better for the study of spermatogenesis than the old.

Since the picric acid must be washed out, fresh 75 per cent alcohol to which a few drops of a saturated aqueous solution of lithium carbonate has been added is placed on the tissue in the supply bottle, and the flow of air started very slowly. By agitation and constant drainage, the time for washing out picric is lessened to one-third that required when the tissue simply soaks in the same solution, which is renewed from time to time. Very small pieces will wash in a few hours. Large pieces must be left until no yellow appears in the fluid.

It is desirable to keep the tissue in alcohol as short a time as possible. As noted in table 3, the shrinkage that occurs in getting the tissue into this grade of alcohol is considerably more than occurred in the fixative.

The anilin oil is started dropping slowly. It is heavier than alcohol so that a stronger current of air may be needed to insure quick mixing. The eye will readily detect the flow of air necessary. When nearly pure anilin is reached, the tissue is left in pure anilin until it is clear like amber.

Clearing. The oil follows by the same method. The tissue should be changed once after being placed in the pure oil, as it is quite essential that all of the anilin be removed, or poor infiltration by paraffin results.

Infiltration with paraffin. This is accomplished by warming the oil and tissue slightly and adding to it every ten minutes a few drops of melted paraffin, which is then thoroughly mixed with the oil by a pipette. This is continued until the mixture is 85 per cent or 90 per cent paraffin. It is then transferred to the melted paraffin. I use one melting point only—52° or 55°C.

If bergamot oil has been used, at least four changes of paraffin should be made, the tissue remaining in each a half hour, and in the fifth paraffin at least one hour. Testis requires longer. I cannot see that twelve to twenty hours in paraffin of 55°C. injures brain or testis.

Imbedding. This is done in the usual way.

RESULTS OF THE METHOD DEVELOPED

Cytological. As stated in the introduction, the object of the experiments was to obtain a method which would positively differentiate dividing cells in the brain of the albino rat. This object was attained. In addition, the same method has given the most satisfactory preparation of the testis yet obtained for the study of spermatogenesis, as simultaneously with the work on the brain tissue the testis was also tested. On this latter material cold Flemming's fluid (0°C.) to which was added 2 or 3 per cent urea gave some very admirable results, but of many testes fixed only two came out well, and these were good only in a few places, while the tubules that showed chromosomes in good condition for study were invariably badly torn, as is illustrated in figures 13 and 15. Figure 10 was made in order to show that this torn condition is general and not confined to one or two places. All stages of spermatogenesis are shown in the tubules of figure 10. It is evident that any conclusions relative to chromosomes drawn from tissues so badly distorted should be fully controlled by a preparation in which no such distortion is to be seen. It is believed that figure 14 illustrates such a preparation.

In the Flemming-fixed material, the early prophases seem more sharply defined, as if the chromatin were compressed into more of a dense thread than in the picro-formol-acetic preparations. It is likely that the latter fixation preserves them in a more nearly normal condition. When chromic acid and urea are added to the picro-formol-acetic mixture the metaphase chromosomes of both spermatocytes are held in some way from clumping, just as they are in the best Flemming preparations, while the prophase threads are not hardened down appreciably. The chromatin maintains its diffuse character between the chromomeres. It may be that on this account the study of these prophase chromosomes will be easier in Flemming-fixed material if one desires to differentiate a single chromosome in the large number possessed by rat and some other mammals, but for the study of the structure of the individual chromosomes, it would seem that the

B-15 preparation is decidedly preferable. I shall have more to say upon this subject in the paper bearing upon the spermatogenesis of the albino rat.

In this paper, however, I wish merely to show the possibilities of isolating chromosomes in the metaphases of spermatocytes by the addition of urea to Flemming's fluid and fixing at 0°C. Figure 15 is to be contrasted with figure 16, where the chromosomes are so clumped as to be hopelessly unresolvable with the best microscopic combinations available. Figure 17, prepared by the B-15 fixative, does not show its full worth in this figure. I had no slide of that preparation which showed several metaphase conditions in polar view, and was compelled consequently to use lateral views at this magnification, trusting that the higher magnification shown in figures 14 and 14 a would bear witness to the real excellence of the preparation. Furthermore only a few cells in figure 17 focussed at the same level, while many did in figure 15. The excellence of general fixation comes out clearly in both figures 14 and 17.

In this connection, attention is called to the apparent excellence of fixation in figure 11. This contrasts favorably with figure 10. Examination of figure 16, however, which is made from the same slide as figure 11, shows the clumped condition of the chromosomes and some spaces about the cells. Neither of these conditions is manifest in figure 17. The spaces near the center of this last figure are the normal lumen of the tubule.

Consider now the cytological details of the brain tissue as illustrated in the figures. The superiority of the general fixation shown in figures 3 and 4 over that shown in figure 2 is manifest. The spaces about the cells in figure 2 do not appear in tissue fixed by B-15 and subsequently treated by the method described in this paper. Tissue fixed in Carnoy's fluid, in formalin, in alcohol and in Ohlmacher's fluid show these spaces throughout. Figures 3 and 4 are typical of the preparations by the method which I have employed in connection with the picroformol-chrome-acetic-urea fixative. No such spaces appear about cells of any size. Large cells have been purposely chosen for the figures, as they would show such spaces most strikingly if present.

In these cells the characteristic fibrillation of the larger protoplasmic process appears distinctly. Fibers are also distinct between the cell bodies. The chromatin masses in the nucleus are more delicately treated than in the other fixations.

The equal action of B-15 throughout the tissue is illustrated in figures 6 and 7. The latter figure shows the outer part of the external granule layer, or where this layer lies on the outside of the cerebellum, while figure 6 shows the same layer deep within a sulcus, as at *I*, figure 1. The cells in each region seem alike. Those in figure 9, on the contrary, are markedly different from those in figure 8. Figure 9 shows the outer cells and figure 8 the deep-lying ones. The appearance in figure 9 is characteristic of Carnoy, alcohol and Ohlmacher preparations, which therefore prove very unsatisfactory fixatives if one desires to study this layer of cells throughout.

The demonstration of dividing cells in photographs was found very difficult for several reasons. One is that the chromosomes are very small and numerous, and that they do not form a flat equatorial plate. A photograph of any equatorial plate would therefore appear to show some fusing. In fact, if one wishes to be absolutely sure of the number of chromosomes, the cell must be slightly inclined to the plane of section, since in a strict polar view one or more of the chromosomes may be entirely hidden by another chromosome. Figure 5 shows a dividing cell in early anaphase, in which some of the chromosomes may be seen separately at this focus. In the preparation they are perfectly distinct from each other. In figure 6, one of the daughter nuclei of a dividing cell in late anaphase is seen (*D. c.* in lower part). This stage is the most critical one in the fusing of the chromosomes. In all other fixatives they mass into a solid body at this stage. In this figure, however, they will be seen as distinct, although too small to be isolated in the photograph. The dividing cell in the upper part of this figure is out of focus, but it can be seen that its edge is not as hard and smooth as the dividing cell in figure 8, which is typical of all other fixatives except B-15.

SHRINKAGE DUE TO FIXATION

Several brains were weighed to show the change in volume due to fixation. The data appear in table 3. The method of weighing is as follows: as soon as the brain is removed it is dipped for a few seconds into the fixative, then removed and drained on filter paper. It is then placed in a weighing bottle and the weight of brain and bottle determined. The brain is then removed and

TABLE 3

Showing loss in weight of different-aged brains in the fixing fluid which gave the best results, viz., picro-formol chrome-acetic urea. See table 1, no. 15

NUMBER OF RAT	SEX	AGE IN DAYS AFTER BIRTH	NUMBER OF HOURS IN FIXING FLUID	WEIGHT IN GRAMS BEFORE FIXATION	WEIGHT IN GRAMS AFTER FIXATION	LOSS OF WEIGHT IN FIXING FLUID	PERCENT-AGE LOSS OF WEIGHT IN FIXING FLUID
133	♂	2	1	0.2860	0.2640	0.0220	7.6
119	♀	3	1	0.2992	0.2770	0.0221	7.4
117	♂	7	1	0.6605	0.6024	0.0581	8.8
118	♀	7	1	0.6799	0.6206	0.0593	8.7
135	♂	8	1	0.9433	0.8969	0.0464	4.9
136	♀	8	1	0.9271	0.8871	0.0400	4.3
137	♂	25	1 $\frac{3}{4}$	1.3590	1.2759	0.0831	6.1
138	♀	25	1 $\frac{1}{2}$	1.2848	1.2031	0.0817	6.4
139	♂	90	2	1.7927	1.6598	0.1329	7.4
139			48	1.7927	1.4398	0.3529	19.6
140	♂	63	2	1.6050	1.4852	0.1198	7.4
140			48	1.6050	1.3611	0.2449	15.2

the bottle weighed before cleaning. The difference between the first and second weights is taken as the brain weight. If the brain is weighed as it comes from the animal, its condition is not the same after being in the fixing fluid, as some of the fluid will remain on its surface. This may be sufficient, even after draining, to counterbalance the loss of weight due to the action of the fixative.

The specific gravity of B-15 is about that of the brain. Any loss of weight while the tissue is in the fluid is therefore due to loss of water. King ('10) found that the loss of weight during fixation in Ohlmacher's fluid varied from 8 per cent to 15 per cent, when left in the fixing fluid from 1 to 6 hours. The 8 per cent

loss was on the brain left in 6 hours. She concludes that length of time in the Ohlmacher fixing fluid does not make much difference in the shrinkage. Her method of calculation, however, was to weigh the brain after fixation and compare this weight with the computed weight for a rat of this size and body weight.

The percentage losses of weight shown in table 3 run from 4.3 to 8.8 per cent when the tissue is left in the fixing fluid one to two hours, but jump to as high as 19.6 per cent when left in 48 hours. Age seems to make little difference, if any, when fixation time remains the same.

SHRINKAGE DUE TO ALCOHOL

It is well known that alcohol shrinks tissue. Table 4 shows that this is considerable if the tissue remains in the alcohol long, running as high as 17.6 per cent when left in 48 hours. These figures show the desirability of passing the tissue through alcohol as quickly as possible.

Table 4 shows also the total loss in weight due to fixing fluid and alcohol up to 60 per cent. These figures run on the whole a little less than those given by King ('10) for Ohlmacher fixation followed by 48 hours in 70 per cent alcohol, these running from

TABLE 4

Showing typical losses in weight of brains in alcohol after fixation as described in table 3, and the total loss of weight due to the effects of the fixing fluid and the alcohol, this loss increasing with the length of time the tissue is left in each fluid

NUMBER OF RAT	SEX	AGE IN DAYS AFTER BIRTH	NUMBER OF HOURS IN ALCOHOL	WEIGHT IN GRAMS WHEN THE ALCOHOL WAS STARTED DROPPING	WEIGHT AT END OF THE TIME INDICATED IN COLUMN 4	LOSS OF WEIGHT	PERCENTAGE LOSS OF WEIGHT	TOTAL LOSS OF WEIGHT IN FIXING FLUID AND ALCOHOL (60 PER CENT)	TOTAL PERCENTAGE LOSS OF WEIGHT
133	♂	2	2	0.2640	0.2360	0.0280	10.6	0.0500	17.4
133			48	0.2640	0.2294	0.0346	13.0	0.0666	23.6
135	♂	8	2	0.8969	0.8780	0.0189	2.1	0.0650	6.9
135			48	0.8969	0.7458	0.1511	16.8	0.0975	20.7
136	♀	8	2	0.8871	0.8664	0.0207	2.3	0.0607	6.5
136			48	0.8871	0.7309	0.1562	17.6	0.1962	21.1
137	♂	25	48	1.2759	1.1046	0.1713	13.6	0.2544	18.7
138	♀	25	48	1.2031	1.0145	0.1886	15.6	0.2703	21.1

16 per cent to 30 per cent, but most of them being in the neighborhood of 21 per cent. There is probably, then, very little difference, if any, between the shrinkage effects of these two fixatives and methods of preparation up to 70 per cent alcohol.

Some rough areal measurements of mesial views of brains indicate that the chief shrinkage is due to alcohol in the method described in this paper, taking the whole technique from fixation through paraffin to the slide. Some substance for replacing alcohol is therefore a decided need in technique.

CONCLUDING REMARKS

I have searched the literature for some mention of the use of chromic acid with a picro-formol-acetic combination, but find none. It would seem that my special contribution to microscopic technique as a result of these experiments is the fixative and the method of agitating fluids automatically as described in this paper.

I am greatly indebted to Professor McClung for constant criticism and advice; also for as constant encouragement when attainment of the desired result seemed hopeless. From him came the suggestion to add to the fixatives some urea, which had proved efficacious in some experiments carried on by Miss Eleanor Carothers at his direction. As an account of these experiments will be printed later by Professor McClung, no further reference need be made in this paper.

The Zoological Department of the University of Pennsylvania has provided me with laboratory facilities at Woods Hole and has placed its exceptional photographic facilities in the University at my disposal.

Professor Donaldson has shown a cordial interest in this work and his encouragement and advice have been very helpful. Wistar Institute has supplied the rats, the number of which passed the hundred mark, and has also provided equipment for carrying on these experiments.

GENERAL CONCLUSIONS ON TECHNIQUE

For cytological work, the slightest gain at any point in the technique is worth working for.

Very gradual change of fluids, agitation of the fluids during changing, and slow infiltration appear essential in order to get the best results from any fixative.

The addition of a low percentage of urea to fixing fluids results in sharpening the chromosomes and preserving the structure of the achromatic nuclear material. It may help penetration of the fluid.

Picro-formol-acetic mixtures are more effective when used at about 38°C. Cold is detrimental.

Flemming's fluid is more effective if used at 0°C. or a few degrees lower.

Flemming's fluid is of no value as a brain fixative at any temperature. It at times (if urea is added) isolates metaphase and anaphase chromosomes in spermatocytes somewhat better than any other fixative tried (except B-15), but is hard on the rest of the tissue and shrinks heavily.

Anilin oil is an excellent substitute for the higher alcohols.

Xylol shrinks tissue more than the vegetable oils.

Special conclusions applicable to the albino rat

B-15 is the only fixative tried which clearly differentiates brain chromosomes. It is superior to all others as a general fixative for developing and adult testis and central nerve tissue. It insures cytological detail in the testis better than any other.

LITERATURE CITED

- ALLEN, EZRA 1912 The cessation of mitosis in the central nervous system of the albino rat. *Jour. Comp. Neur.*, vol. 22, pp. 547-568.
- KING, H. D. 1910 The effects of various fixatives on the brain of the albino rat, with an account of a method of preparing this material for a study of the cells of the cortex. *Anat. Rec.*, vol. 4, pp. 213-244.
- LEE, A. B. 1913 *Microtomists' Vade Mecum*.
- McCLENDON, J. F. 1913 Preparation of material for histology and embryology. *Anat. Rec.*, vol. 7, pp. 51-59.
- SUCHANNEK, 1890 Technische Notiz betreffend die Verwendung des Anilinoels in der Mikroskopie sowie einige Bemerkungen zur Paraffineinbettung. *Zeit. f. w. Mikr.*, Bd. 7, pp. 156-60.

EXPLANATION OF PLATES

The figures are photographed from material fixed in five different ways. They show the essential differences between poor and good preparations.

Plate 1 illustrates the effects of fixatives upon the brain: Plate 2, upon the testis.

Modes of preparation. Figures 1, 3 to 7, 12, 14 and 17 were prepared by the method described in this paper. Figure 2 was fixed in Carnoy's fluid plus 1 per cent chromic acid and 2 per cent urea (table 2, no. 15), and carried through by the method described herewith. Figures 8 and 9 are from tissue fixed in Carnoy's fluid, dehydrated and double-imbedded as described by King ('10). Figures 10, 13 and 15 are from tissue fixed in Flemming's fluid and carried up by the method described in this paper. Figures 11 and 16 are from tissue fixed in Bouin's fluid and carried through by the method described in this paper. Sections were cut at 6 to 10 micra and stained with Iron Haem. and Or. G.

Photographing. Figures 1, 10, 11 and 12 were made with low-power projection lenses; figures 15, 16 and 17, with Zeiss 4 mm. apoc. obj. and Zeiss No. 4 projection ocular; figures 2 to 9, 13 and 14 were made with Zeiss 2 mm. oil immersion apoc. obj. and Zeiss No. 4 projection ocular. All were reduced for publication to the magnifications indicated below.

PLATE 1

EXPLANATION OF FIGURES

1 Sagittal section through cerebellum and medulla of 7-day rat. *Ext. gr. layer* is the region of actively dividing cells, magnified portions of which appear in figures 5 to 9. Only a small portion of the medulla is shown. $\times 15$.

2, 3 and 4 show typical effects of different fixation upon cells in the medulla. Tissue is from 7-day rats. $\times 600$.

2 Fixation: Carnoy plus 1 per cent chromic acid and 2 per cent urea.

3 Fixation: Picro-formol chrome-acetic in urea ('B-15').

4 Fixation: same as figure 3.

5 Dividing cell at inner edge of external granule layer of 7-day cerebellum shown in figure 1. Fixation by B-15. $\times 1200$.

6 to 9 illustrate the external granule layer of the cerebellum of 7-day rat under different fixatives. The contrast is between the surface and the deep-lying portions. $\times 600$.

6 Deep-lying tissue. Fixation: B-15.

7 Surface layer. Fixation: B-15. From same section as figure 6.

8 Deep-lying tissue. Fixation: Carnoy.

9 Surface layer. Fixation: Carnoy. From same section as figure 8.

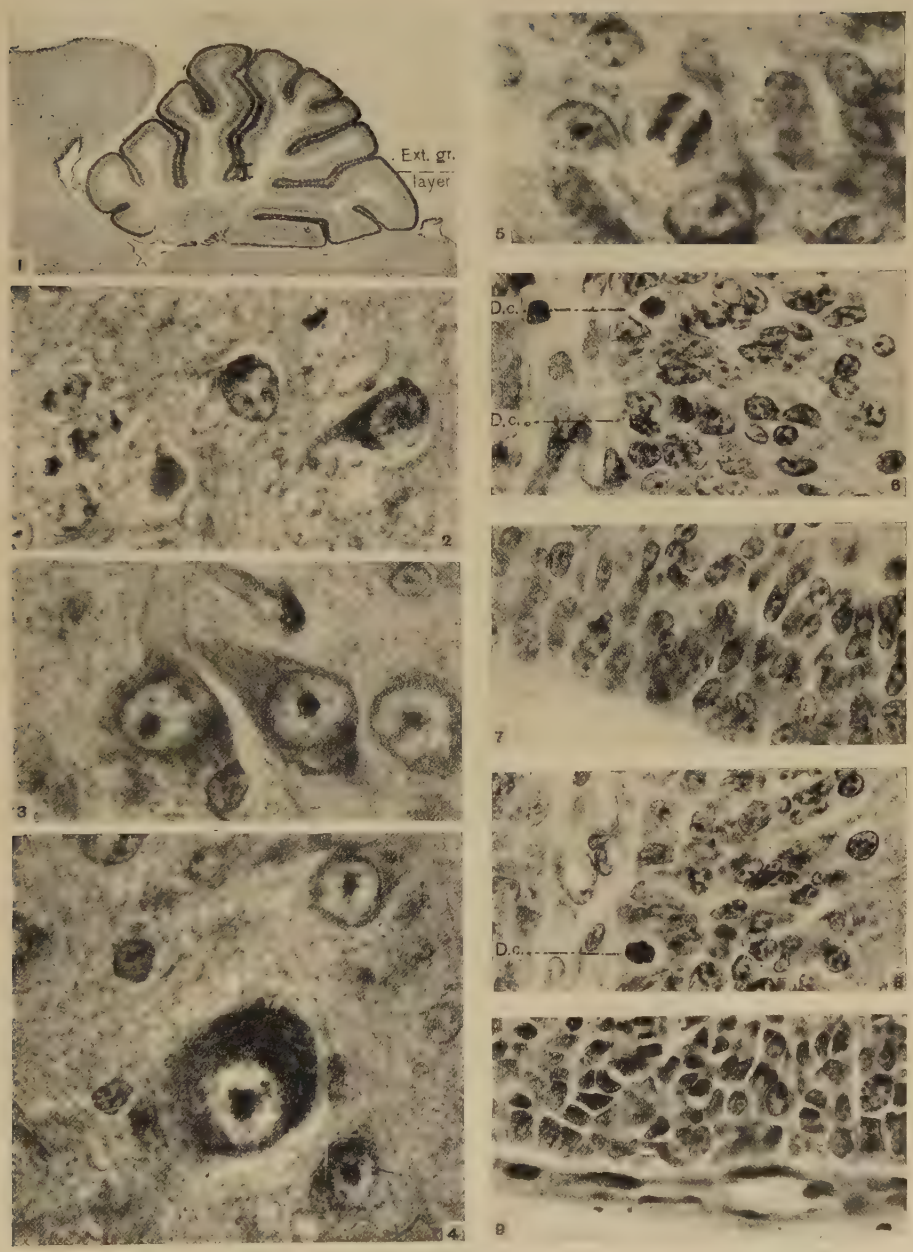
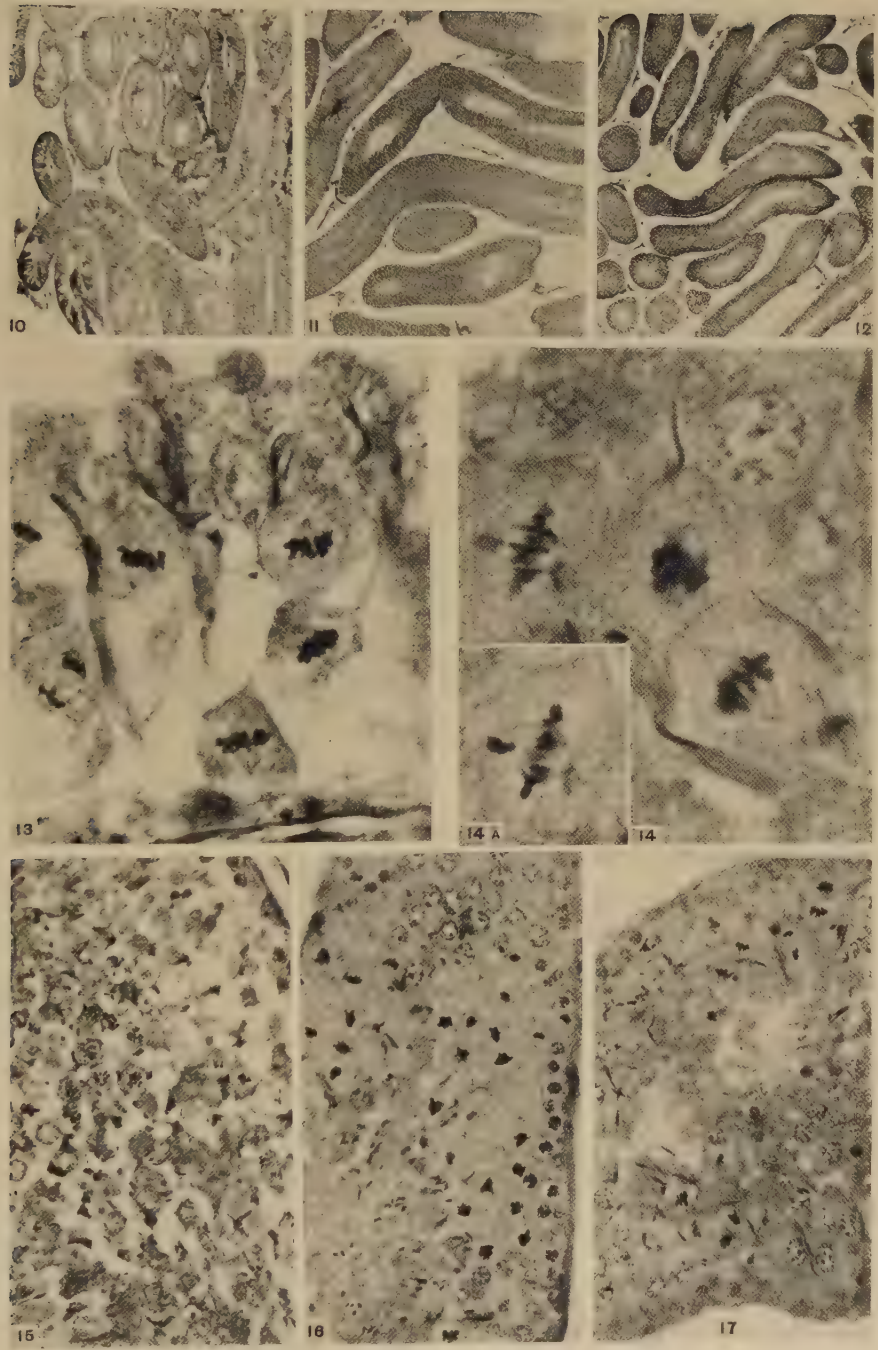


PLATE 2

EXPLANATION OF FIGURES

- 10 Testis fixed in Flemming's fluid. $\times 24$.
- 11 Testis fixed in Bouin's fluid. $\times 24$.
- 12 Testis fixed in B-15. $\times 24$. This tissue is from a younger animal than those shown in figures 10 and 11.
- 13 Same preparation as shown in figure 10. From adjoining slide. $\times 600$.
- 14 From same slide as shown in figure 12. $\times 1200$.
- 14a This figure shows the cell directly above it in figure 14 at a different focus. $\times 1200$.
- 15 From same slide as shown in figure 10. $\times 300$.
- 16 From same slide as shown in figure 11. $\times 300$.
- 17 From same slide as shown in figure 12. $\times 300$. The chromosomes appear at some disadvantage in this figure compared with those in figure 15. The reasons for this are two: (1) they are lateral views in figure 17 as opposed to polar views in figure 15; (2) the cytoplasm is much better preserved in figure 17. A third reason may be suggested: they are less shrunken than in figure 15.



REFERENCE MODELS OF THE ABDOMINAL VISCERA

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THREE FIGURES

It is a common experience that students are frequently handicapped in the study of visceral anatomy by imperfections in the material allotted to them. The occurrence of better viscera on neighboring tables, although contributing alternative objects for study, is often a source of further discouragement.

Quite apart from the numerous cases in which the lungs are more or less disorganized by chronic disease, and the pleurae or peritoneum rendered useless for study by adhesions, there is often difficulty in obtaining sufficiently good preservation. Unfortunately, even after thorough injections with a fluid of good hardening capacity, organs do not become sufficiently resistant to retain their form unless the material is moderately fresh when injected. Delay in the delivery of cadavers not uncommonly leads to practical disintegration of such perishable organs as the pancreas and suprarenals.

A sufficient number of well preserved extra specimens with healthy viscera, prepared in various ways for study, are of great advantage for general reference. Such preparations, with replaceable viscera, etc., are used in many schools, and in a great measure obviate the difficulties above referred to.

We have employed this method, but have abandoned it since the reference preparations require frequent replacement when handled by a large number of students. A sufficient quantity of young or middle aged material, free from chronic disease, is not available for the purpose. During the past few years, we have used casts of the viscera in place of reference preparations, and have found them extremely satisfactory and instructive.

We began by casting the abdominal organs of a well-developed formalin-hardened male who died of double pneumonia at the age of 42. The first casts were made by members of the staff.¹ Later we were fortunate in interesting a very skilled moulder who reproduced our originals and cast the remaining parts directly from our dissections.

¹ Kidney and colon by Mr. J. Globus of Cornell University Medical School; kidney and spleen by Prof. W. C. Smith of Wake Forest Medical College; stomach and liver by Mr. G. Noqué, preparateur in this College; pancreas and duodenum by the writer.

The latter include the bladder, pubic region, and abdominal wall. The complete set consists of all the abdominal organs of this subject with the exception of the jejunum and ileum. We call it "Bellevue Model No. I."

The casts, which are made of a light and durable plaster composition are not used for formal instruction. Two or three sets are constantly in the laboratory, and the students are left to use them as they see fit. We have made it a point to refrain from indicating the uses to which the casts may be put. It has, therefore, amused us somewhat to notice the students at first regarding them with a sort of mild curiosity, and later, as they go deeper into the subject, using them with increasing frequency and interest.

The quality of dissection has by no means deteriorated, in fact it has improved. Reference to the casts, in cases of difficulty, leads to a quicker and surer elementary knowledge of the part dissected and we find that the average student passes naturally to an intelligent understanding of the more interesting and essential relations involved. We have recently used casts of the lungs of a formalin-hardened female. Also a cast of a dissection comprising the diaphragm, the posterior wall of the pericardium, and the respiratory passages, etc., from the same subject. It has seemed to us that these have led to a better understanding of the thorax as a whole.

Hitherto the maker of the casts has sold uncolored sets, at a very moderate price, to several other anatomical departments. These have, in some cases, been painted, as ours have, and appear to have given satisfaction. Experiments in the technique of casting in colors, which are now meeting with success, will soon enable the maker to sell colored casts. These will be about double the price of the uncolored sets but the difficult and unsatisfactory task of painting will be avoided and there will be no tendency, as hitherto, to loss of color from wear.

All inquiries regarding price, etc., should be addressed to this department. Orders should be addressed to G. Von Bouchaute, care of Department of Anatomy, University and Bellevue Hospital Medical College, 338 E. 26th Street, New York.

In making the dissection of the abdominal wall great care was exercised in retaining the movable parts accurately in position. All departures from the common arrangement of structures may be taken as representing those found in the original.

Figures 1, 2 and 3 represent the model reduced about three diameters. The following notes will explain the topography and anomalies of the dissections used for the casts represented.

The transverse section passes through the body of the 10th thoracic vertebra, the 10th costo-vertebral articulations, and the neighboring parts of the 10th ribs; also through the 9th ribs. At the plane of section a right intercostal artery is arising from the aorta, and the vena azygos is receiving a right intercostal vein. The vena hemiazygos and







the thoracic duct are also cut in this section. The longitudinal sections pass through the 9th, 10th and 11th ribs.

The lateral lumbo-costal arch is absent on both sides, leaving an interval between the pars costalis of the diaphragm, and the portion of the pars lumbalis arising from the medial lumbo-costal arch. The anterior surfaces of the 12th ribs appear in the interval.

The ischio-cavernosus and transversus perinei superficialis muscles have been removed from the left side to expose the crus penis and the inferior fascia of the urogenital diaphragm. The superior fascia of the latter structure is convex towards the pelvis, and is seen, perforated for the urethra, between the levatores ani muscles immediately below the arcuate ligament.

The inferior phrenic, the 2nd and 3rd lumbar, and the 12th thoracic arteries are shown on the left side only. There is a communication between the obturator vein, as it is entering the obturator canal, and the external iliac. The 12th intercostal and part of the ascending lumbar vein appear on the left side.

The structure lying between the vena cava inferior and the aorta, opposite the 3rd lumbar vertebra, is a lymph node. A lymphatic vessel passes upward from it to join the cisterna chyli which is hidden by the aorta.

The nerve immediately succeeding the 12th thoracic represents the conjoined ilio-hypogastric and ilio-inguinal, it passes towards the anterior superior spine. The lateral cutaneous nerve of the thigh is more medially placed than usual, and divides into two branches above the anterior inferior spine. The femoral and genito-femoral nerves are conjoined to form a single trunk the origin of which extends from the first lumbar ventral ramus to the fourth. The genito-femoral becomes free just lateral to the place of origin of the inferior epigastric artery. All these nerves are symmetrically placed. The 11th intercostal is shown on the left side only.

A small portion of the sympathetic trunk, and a trunk-ganglion, appears on the left side just medial to the psoas muscle.

THE DEMONSTRATION OF CENTERS OF OSTEOBLASTIC ACTIVITY BY USE OF VITAL DYES OF THE BENZIDENE SERIES

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The sites of osteoblastic activity have hitherto been studied in two ways: by use of microscopic sections, and by the examination of gross specimens in which the areas showing bone formation have been revealed by making the surrounding tissues transparent. Of the so-called 'clearing methods,' the best known and most used are those of Schultze and Spalteholz, the first of which depends for its success on the relative opacity of accumulations of calcium salts, and the latter on the post mortem staining of bone by solutions of alizarin. Therefore they are deficient since they shed no light on the beginning of the ossification process, and they do not make visible the areas in which it is taking place until extensive calcification of the locus has occurred.

If an azo dye (e.g., trypan blue) be administered to a very young animal the bones are stained quickly and very intensely with the vital color. The staining is so rapid and selective that the growing osseous system is colored blue black throughout its entire extent, whereas the organs (the liver, spleen, etc.), which are so deeply stained after the same treatment in the normal adult, are in these young animals in a great measure excluded from participation in the storage of the dye. The bones, if dissected out and cleared, show distinct epiphyses and apophyses sharply outlined within their cartilaginous capsules, and separated by intervening cartilage from the diaphyses. In preparations of this kind, the various centers of osseous development may be readily seen and studied.

The method pursued to attain this end is briefly as follows: Young animals are given a single large dose of a sterile 1 per cent solution of trypan blue. The site of election for the exhibition of the dye is the peritoneal cavity, because the superficial veins are very tiny, and the subcutaneous injection of large amounts of fluid favors pressure necrosis. That the different stages in the process of ossification may be as sharply cut as possible, it is best to administer a large quantity of trypan blue solution at once, rather than to give the same amount of dye in divided doses. Concentrated solutions should be given to avoid the mechanical difficulties involved in the administration of large quantities of fluid. The animal is sacrificed forty-eight hours after staining and the tissues are fixed by injection of a 10 per cent solution of neu-

tral formalin through the blood vessels, followed by immersion in the same solution for a period of from twenty-four to forty-eight hours. The bones are then washed thoroughly, and hardened in ascending grades of alcohol, after which the soft parts are dissected away. The bones are now dehydrated in absolute alcohol, and, after immersion in benzol for twenty-four hours, are cleared in oil of wintergreen according to the method of Spalteholz. Oil of wintergreen is an excellent clearing agent and causes little or no shrinkage in the tissues even after long immersion in the fluid. These preparations are conveniently studied with the aid of the dissecting or binocular microscope.

Bones which have been decalcified previous to clearing may be used for study in the same way, and afterward embedded in paraffin directly from oil of wintergreen and sectioned; or bones previously cleared without decalcification may be returned through absolute to 95 per cent alcohol, decalcified and rehydrated, embedded and cut.

In bones which have been decalcified before clearing, the osteogenetic foci are as distinct though not as opaque as in bones which have not been treated with acid. The loss in opacity is due to the extraction of a small amount of stain which goes hand in hand with the solution of the calcium salts. It is sometimes of advantage in these specimens to introduce as a contrast a very light eosin stain.

The pictures seen in examining these bones are very beautiful. The epiphyseal and apophyseal centers, colored deep blue, are seen embedded in their transparent cartilaginous matrices, separated from the growing end of the diaphysis by a disc of modified cartilage which is readily recognized by the striated appearance which is given it by the arrangement of its cells in parallel columns. The growing ends of the diaphysis are much more darkly stained than the remainder of the bone's shaft; this phenomenon can be readily seen in the advancing ventral ends of the ribs and in the ends of the digital diaphyses adjacent to the epiphyses.

It may be well to emphasize here the fact that stained areas in these bones are not necessarily the so-called primary ossification centers. The stain is deposited in the sites, where, at the time of the exhibition of the dye, active ossification is in progress, and these loci of osteoblastic activity may or may not be identical with the ossification center of the bone or epiphysis under observation. Nowhere is the difference between the primary ossification center and the focus of active growth brought so sharply to the attention of the observer as in the membrane bones of the skull. If one of these bones is examined, it is apparent even to the unaided eye that the vital stain is deposited in a heavy line along the rapidly growing edge of the bone, while the primary ossification centers are seen to be almost entirely without color.

The unique feature of this method is that it demonstrates clearly the different ossification areas even before the process of deposition of calcium salts has occurred in any marked degree. In centers where, by the older methods, the new bone is almost or quite invisible (on account

of the slight amount of calcium salt present) this method brings out the locus of ossific activity very distinctly.

A large field for the application of this method is suggested by the appearance of the bones which have been examined. In the skull, for instance, graphic demonstrations of the growth of membrane bones and closure of the sutures are afforded. In the base of the skull the various anlagen are easily separated through the sharpness of the contrast between the stained new bone and the intervening masses of cartilage. In the long bones, the epiphyses and apophyses stand out with diagrammatic clearness.

The physiological and histological aspects of the vital staining of bone with trypan blue are now being considered, and will be published in the near future.

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